Acta Biomaterialia 49 (2017) 260–271

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Development, fabrication and evaluation of a novel biomimetic human breast tissue derived breast implant surface

S. Barr [a,b], E.W. Hill [b], A. Bayat [a,c,*]

[a] Plastic and Reconstructive Surgery Research, Stopford Building, Oxford Road, University of Manchester, Manchester M13 9PT, UK
[b] Department of Computer Sciences, University of Manchester, Manchester M13 9PL, UK
[c] Bioengineering Research Group, School of Materials, Faculty of Engineering & Physical Sciences, The University of Manchester, Manchester, UK

ARTICLE INFO

Article history:
Received 28 August 2016
Received in revised form 18 November 2016
Accepted 22 November 2016
Available online 2 December 2016

Keywords:
Breast implant
Capsular contracture
Biomaterial
Novel surface development
Biocompatibility
Biomimetics

ABSTRACT

Breast implant use has tripled in the last decade with over 320,000 breast implant based reconstructions and augmentations performed in the US per annum. Unfortunately a considerable number of women will experience capsular contracture, the irrepressible and disfiguring, tightening and hardening of the fibrous capsule that envelops the implant. Functionalising implant surfaces with biocompatible tissue-specific textures may improve *in vivo* performance. A novel biomimetic breast implant is presented here with anti-inflammatory *in vitro* abilities.

Topographical assessment of native breast tissue facilitated the development of a statistical model of adipose tissue. 3D grayscale photolithography and ion etching were combined to successfully replicate a surface modelled upon the statistics of breast tissue.

Pro-inflammatory genes ILβ1, TNFα, and IL6 were downregulated ($p < 0.001$) and anti-inflammatory gene IL-10 were upregulated on the novel surface. Pro-inflammatory cytokines Gro-Alpha, TNFα and neutrophil chemoattractant IL8 were produced in lower quantities and anti-inflammatory IL-10 in higher quantities in culture with the novel surface ($p < 0.01$). Immunocytochemistry and SEM demonstrated favourable fibroblast and macrophage responses to these novel surfaces.

This study describes the first biomimetic breast tissue derived breast implant surface. Our findings attest to its potential translational ability to reduce the inflammatory phase of the implant driven foreign body reaction.

Statement of Significance

Breast implants are still manufactured using outdated techniques and have changed little since their inception in the 1960's. Breast implants can cause a medical condition, capsular contracture which often results in disfigurement, pain, implant removal and further surgery. This condition is due to the body's reaction to these breast implants. This article describes the successful development and testing of a novel breast implant surface inspired by the native shapes present in breast tissue. Results show that this novel implant surface is capable of reducing the negative reaction of human cells to these surfaces which may help reduce capsular contracture formation. This work represents the first steps in producing a biocompatible breast implant.

1. Introduction

Breast Implant based surgery is performed in a variety of settings, from reconstruction for congenital anomalies and post-mastectomy defects, to augmentations for cosmetic purposes. 1,773,584 breast augmentations for aesthetic reasons were declared worldwide to the International Society of Aesthetic Plastic Surgery in 2014 [1]. Breast implants are not without their inherent complications, with the pathological contracture of the capsule being the most frequent and predominant cause for patient dissatisfaction post-implantation [2,3]. Contracture rates occur in a not insignificant 17.5% of implant based procedures [4].

Capsular contracture is the pathological fibrous amplification of the foreign body response to the breast implant. Typical progres-

* Corresponding author at: Plastic and Reconstructive Surgery Research, Stopford Building, Oxford Road, University of Manchester, Manchester M13 9PT, UK.
E-mail address: ardeshir.bayat@manchester.ac.uk (A. Bayat).

http://dx.doi.org/10.1016/j.actbio.2016.11.052
1742-7061/

sion of the foreign body reaction results in a functional capsule, which helps to maintain implant position [5]. In contracted capsules, this response is exaggerated and the capsule becomes thickened, fibrotic and less pliable, which can manifest as mastalgia, breast firmness and a poor aesthetic result which often culminates in re-operation [6].

Capsular contracture is a multifactorial condition. Filler material, sub-muscular placement of the implant, adjuvant radiotherapy and bacterial colonisation have been implicated in its development [7]. Recently a growing body of evidence has linked biofilm formation with capsular contracture [8]. Sterile precautions have proved important in reducing capsular contracture rates [9]. Capsular contracture has also been linked to implant surface texture, the focus of this paper.

Current breast implants, with an elastomer shell and saline or silicone gel filler are derived from a 1960′s design, which evolved into a textured, polyurethane coated implant in the late 1960′s [10,11]. Concerns that polyurethane was pro-cancerous led to a moratorium on this surface in the US market. Recognising the ability of polyurethane to alleviate contracture rates and due to a conceived perception that this was a consequence of implant texture, several silicone implant surface textures, which attempted to mimic the polyurethane surface were developed [12,13]. Implant textures that are currently available are manufactured by either imprinting salt or polyurethane foam into the uncured implant shell or by moulding the implant surface from a pre-textured, often sandblasted mould [14]. Whilst the manufacturing techniques employed are crude, their development is constrained by a litigious legacy and the textures produced remain poorly characterised. Recent systematic review and a meta-analysis, have both demonstrated the protective effect of implant texture on capsular contracture [15–17]. No study has managed to standardise the confounding variables of capsular contracture and demonstrate that one particular implant surface is most efficient at reducing this condition.

Biological inspiration has been utilised in many branches of engineering to elucidate new approaches to technical problems; biomimicry is the extension of this principal [18]. Biological surfaces have evolved over millions of years to produce materials and topographies that are highly organised from the macro- to the nano-scale. Micro and Nano surface topographies have been shown to influence cell proliferation, attachment, adhesion, migration and morphology [19]. Many of the morphological topographies, which exist *in vivo* that interact with cells are those from the extra cellular matrix (ECM). The ECM of different tissue types promote the production of distinct tissue morphologies from where they are derived [20,21]. Surfaces that mimic the physiological ECM have been theorised to decrease the foreign body reaction and modulate the immune response, improving the bodies reaction to these surfaces [22].

Breast implants are frequently inserted into the sub-glandular tissue plane, between the adipose tissue of the breast anteriorly and the pectoralis muscle fascia posteriorly. In this study adipose tissue was isolated from this tissue interface to generate a novel surface, the "original adipose" surface. This surface represents a tissue specific extension of a recent publication by another group member, Kyle et al. who generated an acellular dermal matrix inspired surface [23].

In the process of achieving this novel adipose surface, it was recognised that breast tissue has an inherent, measurable surface structure that was modelled and generated to produce a secondary "modelled adipose" surface. Nano-technology has largely unaffected the development of breast implants and thus the aim of this study was to integrate nano-technological approaches with biological inspiration [24]. The rationale behind this approach was that these textures could promote responses from cells important in dictating the foreign body response to the implant at a nano and micro scale, whilst also providing a surface which was comprised of textures with larger tissue dimensions capable of integrating with breast tissue.

To assess the ability of these surfaces to mediate the foreign body and inflammatory response in an *in vitro* environment macrophages, the cell type shown to have the most powerful effect on the processes of tissue repair, remodelling and biocompatibility were utilised [25]. THP-1 human macrophages were cultured on the novel surfaces developed and their morphology, gene expression and cytokine secretions quantified to ascertain the effects of these surfaces [26].

The ultimate aim of this study therefore was to produce a site-specific, hierarchically textured implant topography with provenance from the breast.

2. Materials and methods

This article describes the characterisation and fabrication of two novel polydimethysiloxane (PDMS) implant surfaces derived from native breast tissue topography; the original adipose and the modelled adipose surface. Tissue samples used in this study were obtained through the Plastics and Reconstructive Surgery Research (PRSR) Skin and Tissue Bank ethics (North West Research Ethics Committee Ethics Code – 11/NW/0683 Date: 2/11/2011). Informed consent was obtained from patients for the use of their tissue in this study. All breast tissue processing was done at a Human Tissue Authority licensed laboratory.

2.1. Collection of breast tissue and sample fixation

Breast tissue from three patients was collected from elective cosmetic breast reduction operations and transferred to the laboratory in Dulbecco's Modified Eagle Medium (Sigma-Aldrich, UK) supplemented with 1% penicillin and streptomycin (PAA laboratories, Pasching, Austria), 1% L-glutamine (PAA) and 10% Fetal Bovine Serum (PAA). Patients had no past medical history of any malignancy or fibrotic conditions, none were obese and none smoked. Patient demographics are included in Supplementary Table 1.

Breast tissue was washed thoroughly in warmed phosphate buffered saline (PAA) supplemented with 1% Penicillin and Streptomycin (PAA) before the lobules of breast adipose tissue were dissected. Breast adipose tissue was fixed in paraformaldehyde 2% (Sigma-Aldrich), glutaraldehyde 2.5% (Sigma-Aldrich) and 0.1 M hepes buffer (Formedium, UK) for 7 days at 40 °C.

Adipose tissue was washed four times in distilled water for 15 min each and then post fixed in osmium tetroxide 1% (Agar Scientific, UK) in 0.1 M hepes (Formedium, UK) for 1 h. Following two further wash steps in distilled water of 15 min each, the tissue was dehydrated using graded acetone steps of 25%, 50%, 75%, 90%, and 100%, for 15 min at each step. Three further washes in 100% acetone were then performed before the tissue was critical point dried (Quorum Technologies Ltd., UK).

2.2. Imaging, sample measurement and generation of original adipose surface

Fixed adipose tissue was mounted on a scanning electron microscopy (SEM) stub and scanned using an X-100/X-200 series 3D laser confocal microscope with a 50× objective (Keyence, Japan). SEM mounted samples were sputter coated with gold and palladium for 120 s using a SC7620 sputter coater (Quorum Technologies Ltd, UK) and imaged using an Quanta 250 FEG SEM (FEI, Oregon, USA). Images from the laser confocal microscope were then exported as an .asc point group data file. This .asc file was